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A NOVEL APPROACH TO PHOSPHOPEPTIDE SYNTHESIS-PREPARATION OF GLU-PSER-LEU

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ABSTRACT: A synthesis of the phosphotripeptide Glu-PSer-Leu is reported via the stepwise incorporation of the protected phosphoamino acid N^{α}-tert-butoxycarbonyl-O-dibenzylphosphono-L-serine.

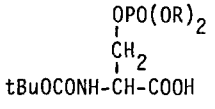
Our interest in the structure and reactivity of the heavily phosphorylated area of the α and β caseins has lead to the choice of the homologous region (1) and its congeners as synthetic targets.

1

We wish to describe in this letter our strategy and methodology for constructing complex phosphorylated sequences through the preparation of the tripeptide, Glu-PSer-Leu (5).

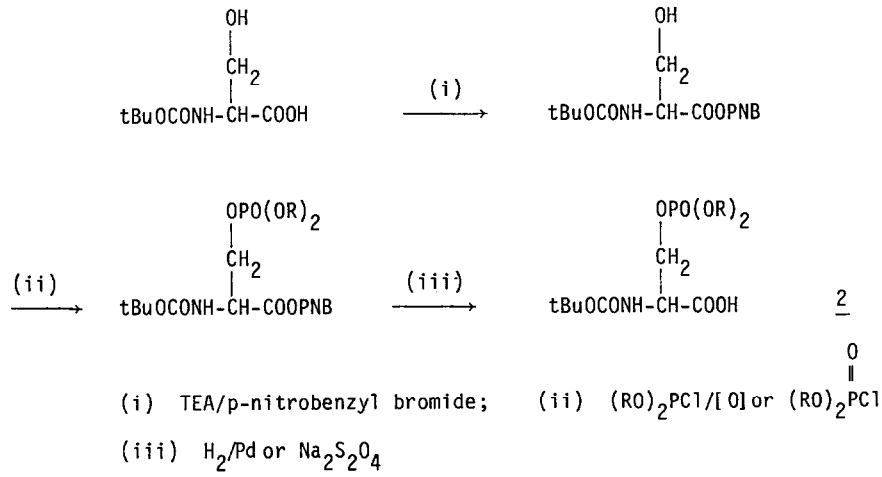
Our initial attempts[1] to generate phosphopeptides using traditional procedures[2] were frustrated by complex mixtures. In our hands, the use of phosphochloridates, $(RO)_2POCl$ as phosphorylating agents for peptide substrates gave either incomplete phosphorylation (for R=Bzl) or partial deprotection on hydrogenation (for $R=C_6H_5$). A more serious limitation to this 'Total Phosphorylation' approach is its incompatibility with the preparation of specifically phosphorylated peptides e.g., -Ser-PSer-Glu-Glu-.

To avoid the difficulties inherent in former approaches we chose the novel strategy which incorporates the phosphate group as a suitably protected phosphoamino acid (2).



2

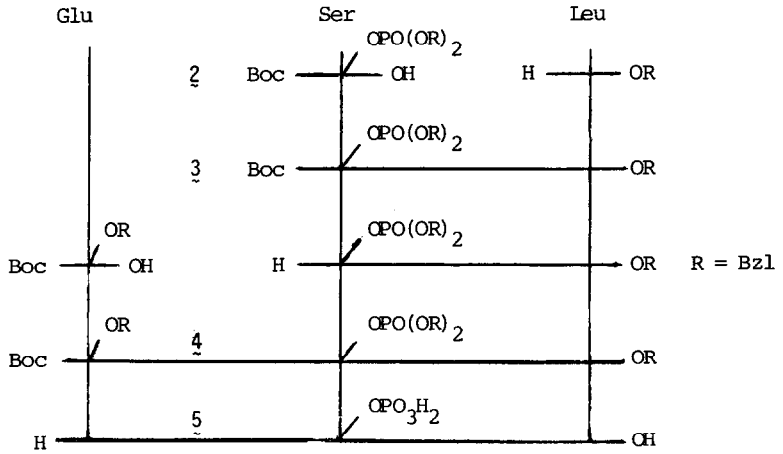
The synthesis of 2 is outlined in Scheme 1 and experimental details are provided elsewhere[3,4].



Scheme 1

We now wish to report the inclusion of 2 (R=Bzl) in the synthetic sequence Glu-PSer-Leu (5) via solution phase methodology. The phosphopeptide segment 5 occurs both in casein and in bovine enamel peptide sequences. These have been postulated to be important Ca^{2+} binding sites, playing roles in micelle stabilization[5] and enamel calcification.

The synthesis of 5 is outlined below (Scheme 2).



Scheme 2

All couplings were performed using the excess mixed anhydride procedure[7,8]. Coupling yields were 95% and 80% respectively for the intermediate protected peptides 3[11] and 4[12] which were homogeneous by tlc criteria. These species were readily characterised by their ^{1}H, ^{13}C and ^{31}P nmr spectra[13,14]. ^{31}P nmr values[15] of -0.9 and -0.8 ppm, typical of phosphotriesters, were observed for peptides 3 and 4, respectively.

'Debocking' of the intermediate peptide 3 was achieved using 98% formic acid. This was necessary as the usual acidolytic reagents (4M HCl/dioxan, CF_3COOH/CH_2Cl_2) caused considerable debenzylation of the phosphotriester functionality, as observed by ^{31}P nmr. We will report on the stability of protected phosphoserine residues incorporated in a peptide chain at a later date.

A one step deprotection of 4 was achieved quantitatively by hydrogenolysis using 10% Pd/C in formic acid. This gave pure 5 as white flakes, $[\alpha]_D^{21}$ - 14.0 (C4, 1M HCl). The HPLC profile (μ Bondapak C18, 0-50% CH_2CN/.1% TEAP pH 4.0, 1ml/min, 214 nm) showed a single peak and ^{1}H, ^{13}C and ^{31}P nmr spectra[16] were assigned and contained no additional contaminant resonances. A satisfactory amino acid analysis was obtained[17].

By several criteria this synthesis has increased our confidence in the incorporation of protected phosphoserine residues into a growing peptide chain. The high coupling yields achieved and the stability of the resultant phosphotriester together with a simple final deprotection step suggest future phosphopeptide syntheses along these lines to be a viable proposition.

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NOTES AND REFERENCES

1. P.F. Alewood, R.B. Johns and J.W. Perich in "Peptides: Proceedings of the Seventh American Peptide Symposium" P. 65, Pierce Chemical Co., Ill. (1981).

2. G. Folsch, *Svensk Kemisk Tidskrift*, 79, 38 (1967).

3. P.F. Alewood, J.W. Perich and R.B. Johns, *Synth. Commun.*, 12, 821 (1982).

4. P.F. Alewood, J.W. Perich and R.B. Johns, *Aust. J. Chem.*, accepted for publication, 1983.

5. D.G. Schmidt and T.A.J. Payens, *Surface and Colloid Science*, 9, 165 (1967).

6. A. Papas, J.M. Seyer and M.J. Glimcher, *FEBS Letters*, 79, 276 (1977).

7. A. van Zon and H.C. Beyerman, *Helv. Chim. Acta*, 59, 1112 (1976).

8. The optical integrity of the phosphoamino acid coupling step was confirmed by the conversion of 3 (H_2, Pd/C, HCOOH) to the known phosphodipeptide PSer-Leu; $[\alpha]_D^{21}$ -15.0 (C4, 1M HCl) lit.[9] $[\alpha]_D^{21}$ -16.0 (C4, 1M HCl); lit.[10] [(d)PSer-Leu], $[\alpha]_D^{21}$ -28.1 (C3.6, 1M HCl).

9. G. Fölsch, *Acta. Chem. Scand.*, 13, 1407 (1959).

10. G. Fölsch, *Acta. Chem. Scand.*, 13, 1422 (1959).

11. In the coupling of synthon 2 to Leu-OBzl, ether was the workup solvent of choice to avoid contamination of the dipeptide 3 with excess sodium salt of 2.

12. 4 required flash chromatography on silica gel due to baseline impurities.

13. ^{13}C nmr $\delta(CDCl_3)$ 3: 171.8, 168.4, 155.0, 135.2 (d, J=5.86Hz) 135.0, 128.2, 127.9, 127.7, 80.0, 69.2 (d, J=5.86Hz), 66.5, 66.5 (unresolved doublet), 54.1 (d, J=5.86Hz), 50.7, 40.8, 27.9, 24.3, 22.4, 21.5, 18.7.

14. ^{13}C nmr $\delta(CDCl_3)$ 4; 173.0, 171.9, 168.1, 155.7, 135.4, 135.3 (d, J=5.86Hz), 135.5, 128.6, 128.2, 128.1, 80.3, 69.6 (d, J=5.86Hz), 66.8, 66.8 (unresolved doublet), 66.7, 54.3, 53.2 (d, J=5.86Hz), 51.2, 40.8, 30.4, 28.3, 27.4, 24.7, 22.8, 21.8.

15. Relative to 85% H_3PO_4.

16. ^{13}C nmr $\delta(H_2O)$ 5; 176.6, 176.6, 170.6, 169.7, 64.5 (d, J=5.86Hz), 54.6 (d, J=5.86Hz), 52.7, 52.1, 39.9, 29.6, 26.3, 24.9, 22.7, 21.1.
^{31}P nmr $\delta(H_2O)$ 5; 1 peak, + 0.3.

17. Amino acid analysis (6M HCl, 105°): Glu 0.97 (1); Ser 0.88 (1); Leu 1.00 (1); Ala 0.01. The presence of Ala arises from β elimination and subsequent hydrogenation of the resulting dehydrotripeptide.

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